

EVALUATION OF SOME MULBERRY SPECIES AND THEIR SUITABILITY FOR PROCESSING JAMS

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ABSTRACT

Three species of mulberry fruits namely white mulberry (*Morus alba* L.), red mulberry (*Morus rubra* L.) and black mulberry (*Morus nigra* L.) cultivated in Egypt as well as the comparison between the antioxidant activity of fresh fruits and jams prepared from selected species were evaluated. Results showed that protein content was 1.38, 1.24 and 1.15%, total sugars 12.75, 15.25 and 16.03% in black, red and white mulberries, respectively. Reducing sugars varied from 11.32 to 14.00% in black, red and white mulberries and total lipids ranged from 1.08 and 1.75% in black and red mulberry fruits. Also, crude fibre was 1.60, 2.17 and 2.28% in three mulberry species while pH value ranged from 5.49 and 5.80. Results also indicated that black mulberry had the highest content of total phenolics, total flavonoids and anthocyanins content otherwise white mulberry had the lowest content. Fractionation of phenolic compounds in all mulberry species represented the highest content of protocatechuic meanwhile both red and white mulberries had moderate contents of p.hydroxybenzoic acid. The mineral analysis showed that potassium was the most abundant element in all three mulberry species. Both black and red mulberries had high calcium content followed by sodium and magnesium. At the same time, red mulberry had the highest content in iron and zinc compared with the other two species. Results also showed that BHT had the highest antioxidant activity compared with fresh mulberry fruits or jams prepared from these fruits (92.95%). Fresh black mulberry had the highest antioxidant activity (86.46%), while fresh red and white mulberries recorded 60.35 and 55.31%, respectively. Jams prepared from three mulberry species gave the lower antioxidant activity. The sensory assessment indicated that jam prepared from black mulberry recorded the best sensory properties followed by red mulberry jam while the lowest score for taste, odor and texture was observed in jam prepared from white mulberry.

Keywords: Mulberry, *Morus alba*, *Morus rubra*, *Morus nigra*, Chemical Characteristics, Jams

INTRODUCTION

The mulberry fruit belongs to the genus *Morus* of the family Moraceae. There are 24 species of *Morus* and one subspecies, with at least 100 known varieties. Mulberries are grown found from temperate to subtropical regions of the Northern hemisphere to the tropics of the Southern hemisphere and they can grow in a wide range of climatic, topographical and soil conditions. (Tutin *et al* 1996).

The three mulberry species (*Morus alba*, *Morus nigra*, *Morus laevigata*) can be seen in different regions, which can taste very pleasant when eaten fresh or transformed into other uses as marmalades, juices, liquors, natural dyes and in the cosmetics industry (Ercisli and Orhan, 2007).

Mulberry fruit is consumed fresh and in dry state and has unique nutritional value among the fruits. Its fruits are juicy and rich in sugar (10%) and iron (2.3 mg/100 g). Also jam, cold beverages and wine are made from it (Singh, 1992).

Recently, red and black mulberries have gained an important position in the food industry due to the presence of anthocyanins. Several researchers have previously reported that anthocyanins have remarkable antioxidant and free-radical scavenging activities (Wang *et al.*, 1997; Stintzing *et al.*, 2002). Additionally, multiple findings suggest that anthocyanin contents of berries and red fruits may provide possible health benefits such as reduced risk of coronary heart disease, stroke, certain types of cancers and aging (Prior, 2003; Zafra-Stone *et al.*, 2007).

The chemical composition of white (*Morus alba* L.), red (*Morus rubra* L.) and black (*Morus nigra* L.) mulberry fruits were studied by Ercisli and Orhan (2007) who found the highest total phenolic and flavonoid contents in black mulberry while the highest total fat content showed in white mulberry. The total soluble solids content and mineral compositions varied in different mulberry species.

Also, Koca *et al* (2008) stated that mulberry is widely consumed fresh as well as dried, processed into jam or juices and they studied, anthocyanin composition, chemical composition and antioxidant activity of wild purple mulberry.

In addition, mulberry fruit is well known as a good source of anthocyanins with many biological activities. However, there are several colors of mulberry fruits, even from the same species. The purple-colored mulberry fruit extract contains the highest levels of anthocyanin and strongest antioxidant activity compared with the other colors of mulberry fruit extracts. Light or heat exposure of the mulberry fruit extract deteriorated total anthocyanin and ascorbic acid content and led to decrease the antioxidant activity (Aramwit *et al* 2010).

Good correlations were observed among the phenolic, anthocyanin, and proanthocyanidin contents and the radical scavenging capacities of mulberry fruits. The high total phenolic content of mulberry fruits were mainly contributed to anthocyanins, rutin, and chlorogenic acid (Isabelle, 2008).

However, studies on characterization and quantification of phytochemical and antioxidant properties of mulberry fruits are very limited. Previously, Naderi *et al* (2004) found that extracts of *M. nigra* fruits have a protective action against peroxidative damage to biomembranes and biomolecules.

Traditionally, deep coloured fruits, vegetables or foods are recognized as more healthy to human body, especially in the oriental countries. There has been a growing interest in pigment components of fruits and vegetables, which may promote human health or lower the risk for disease (Lin and Tang, 2007).

Numerous studies were carried out on plants resulting for in the development of natural antioxidant formulations for food, cosmetic and other applications. In addition to scientific information on antioxidant properties of various plants, Therefore, the assessment of such properties remains an

interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and nutraceuticals (Miliauskas *et al.*, 2004; Arabshahi-Delouee and Urooj, 2007).

Therefore, in the present work, the chemical characteristics of selected three mulberry species cultivated in Egypt were studied. Moreover, mulberry fruit jams prepared from selected fruits were chemically and sensory evaluated. Furthermore, the antioxidant activity of fresh fruits and jams prepared from three mulberry species was compared with that of BHT as synthetic antioxidant.

MATERIALS AND METHODS

Materials:

Three species of mulberry fruit named *Morus alba* L., *Morus rubra* L. and *Morus nigra* L. were obtained from Horticultural Research Institute, Agriculture Research Center Giza, Egypt during 2009 and 2010 years.

2,2-diphenyl-1-picryl hydroxyl (DPPH) used for deracination of total antioxidant activity, Butylated hydroxytoluene (BHT) and phenolic compounds were purchased from Sigma Co.

Methods:

Preparation of mulberry fruit samples

The fruits were immediately transported to the laboratory, packaged in plastic bags, and stored below 4°C in a refrigerator. After the stems were taken away, mulberry fruits were first washed carefully under running tap water, dried. Then, the homogenized fruit sample was weighed and immediately transferred into a beaker for further processing.

Preparation of mulberry fruit jams

Mulberries (400 g), sucrose (366 g) and pectin (1.8 g) were used for the jams preparation of three mulberry species. Citric acid was used for adjusting pH values for proper gelatinization of pectin. Mulberries, larger part of sucrose and citric acid were mixed and cooked. Pectin was mixed with part of sucrose and added at the final stage of the jam cooking. Mulberries jams were cooked until the final product contained 65% of soluble solids (determined by refractometer).

Chemical characteristics

The moisture, ash, total lipids, crude protein (nitrogen x 6.25), crude fibre, total and reducing sugars and ascorbic acid contents were determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 2000).

Total soluble solids were determined by using refractometer at ~ 25°C and the results were expressed as Brix.

Flavonoids were extracted and determined according to Zhuang *et al* (1992).

Total anthocyanins were determined according to the method described by Colin and Peter (1980).

Assay for total phenolics

Total phenolic constituents of mulberry fruits were performed employing the literature methods involving Folin- Ciocalteu reagent and gallic

acid as standard (Slinkard and Singleton, 1997). The extract solution (0.1 ml) containing 1000 mg extract was taken in a volumetric flask, 46 ml distilled water and 1 ml Folin-Ciocalteu reagent was added and the flask shaken thoroughly. After 3 min, 3 ml of solution 2% Na₂CO₃ was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated to all standard gallic acid solutions.

Extraction, separation and quantification of phenolic compounds were determined according to the method described by Goupy *et al* (1999).

Antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was carried out according to the method of Brand-Williams *et al.* (1995) with some modifications by Thaipong *et al* (2006). The solution was prepared by dissolving DPPH (24 mg) in methanol (100 mL) and then stored at (-20 °C) until needed. The solution was obtained by mixing the stock solution (10 mL) with methanol (45 mL) to obtain an absorbance of 0.950 ± 0.02 units at 517 nm using the Jenway 6405 UV/Vis spectrophotometer. The fruit extracts (0.2mL) were allowed to react with the DPPH solution (3 mL) for 1 hour in the dark place. Thereafter, the absorbance was taken at 517 nm. The antioxidant activity was calculated as a decrease in the absorbance value using the formula:

Inhibition activity (%) = (Abs. Control – Abs. Sample/Abs. Control) × 100%. The same procedure was repeated with butylated hydroxytoluene (BHT) at the same concentration and a blank containing only 350 ml of ethanol. After the incubation period the absorbance of the mixtures were measured at 517 nm. Antioxidant activity of the samples were compared with BHT and the blank.

Organoleptic evaluation

Organoleptic evaluation for color, taste, odor, texture and overall acceptability was determined by the method as described by Larmond (1977).

Statistical analysis

All data were recorded as means and analyzed by SPSS Windows (ver.18.). One-way analysis of variance (ANOVA) and Duncan comparisons were tested to signify differences between raw and different varieties of mulberry.

RESULTS AND DISCUSSION

Gross Chemical constituents

Chemical constituents of three mulberry fruit species are given in Table (1). The moisture content varied from 76.53 to 80.30%. The highest percentage was observed in black mulberry (80.30%) followed by red mulberry while the lowest of moisture was noted in white mulberry (76.53%). Protein content was 1.38, 1.24 and 1.15% on fresh weight in black, red and white mulberry, respectively. Also, results show the highest content in total lipids being 1.75% in red mulberry while the lowest percentage was shown in black mulberry (1.08%). The content of total sugars were 12.75, 15.25 and

16.03% in black, red and white mulberries, respectively. Reducing sugars in the three species fresh mulberry fruits varied from 11.32 to 14.00%, red mulberry had the highest percent followed by white mulberry (12.13%) while the lowest reducing sugars were found in black mulberry. Non reducing sugars ranged from 1.43 in black mulberry to 3.90% in white mulberry. Also, crude fibre percentages were 1.60, 2.17 and 2.28% in the three mulberry species while pH values were 5.49, 5.80 and 5.79, respectively.

Table (1): Chemical constituents of three mulberry fruit species (on fresh weight basis)

Chemical constituents (%)	Black mulberry <i>Morus nigra</i> L.	Red mulberry <i>Morus rubra</i> L.	White mulberry <i>Morus alba</i> L.
Moisture	80.30±0.42	78.09±1.46	76.53±1.56
Protein	1.38±0.17	1.24±0.32	1.15±0.14
Total lipids	1.08±0.11	1.75±0.30	1.71±0.15
Ash	1.11±0.15	1.25±0.16	1.27±0.12
Total soluble solids	13.38±0.18	22.25±1.77	19.12±1.23
Reducing sugars	11.32±1.32	14.00±0.69	12.13±0.89
Non-reducing sugars	1.43±0.17	1.25±0.19	3.90±0.28
Total sugars	12.75±0.62	15.25±0.57	16.03±1.02
Crude fibre	1.60±0.41	2.17±0.14	2.28±0.16
pH value	5.49±0.24	5.80±0.22	5.79±0.21

Results are presented as mean value of duplicates Mean values ± Std.Deviation

Ascorbic acid, total phenolics, total flavonoids and total anthocyanins

Ascorbic acid, total phenolics, total flavonoids and total anthocyanins content of the three mulberry species are noticed in Table (2). The results showed that ascorbic acid was 27.75, 29.30 and 30.04 mg/100g in black, red and white mulberries, respectively. The total phenolics and flavonoids contents in different three species varied considerably which were (432.55, 50.12), (217.68, 39.32) and (143.73, 23.22) mg/100g on fresh weight, respectively. This result was less than that obtained by Ercisli, and Orhan (2007). This difference may be due to the change in climatic conditions. Furthermore, anthocyanins content of the three species were 24.65, 17.73 and 1.59mg/100g. Results indicate that the black mulberry had the highest content for total phenolics, total flavonoids and anthocyanin content otherwise white mulberry had the lowest content.

Table (2): Ascorbic acid, total phenolics, total flavonoids and total anthocyanins content of three mulberry fruit species mg/100g (on fresh weight basis)

Phytochemical compounds	Black mulberry <i>Morus nigra</i> L.	Red mulberry <i>Morus rubra</i> L.	White mulberry <i>Morus alba</i> L.
Ascorbic acid	27.75±1.33	29.30±1.68	30.04±1.90
Total phenolics	432.55±8.64	217.68±3.51	143.73±5.12
Total flavonoids	50.12±2.52	39.32±0.83	23.22±1.15
Total anthocyanins	24.65±0.65	17.73±1.24	1.59±0.31

Results are presented as mean value of duplicates Mean values ± Std.Deviation

Fractionation of phenolic compounds in three mulberry fruit species

Phenolic compounds of fresh black, red and white mulberries are represented in Table (3). Eleven phenolic compounds were identified in the three mulberry species. The detected phenolic compounds were

protocatechuic, p.hydroxybenzoic, chlorogenic, caffeic, catechin, syringic, ferrulic, chrysin, cinnamic, p.coumaric and coumarine. Results showed three mulberry species had the highest content of protocatechuic which was 145.79, 56.77 and 41.40 mg/100g (on fresh weight) for black, red and white mulberries, respectively. Meanwhile both red and white mulberries had moderate content of p.hydroxybenzoic which was 19.19 and 15.94 mg/100g, Also, moderate content was observed in caffeic acid in both red and white mulberries. The content of caffeic acid was 12.53 and 10.24mg/100g, respectively. Also, the content of catechin and syringic were 27.73 and 23.69mg/100g in black mulberry while not detected or slightly content in other species. Furthermore, ferrulic, chrysin, cinnamic, p.coumaric and coumarine had low content or not detected in all mulberry species. Zadernowsk *et al* (2005) identified some phenolic compounds in black mulberry such as protocatchoic, caffeic, p.coumaric and ferrulic at different percentages.

Table (3): Phenolic compounds of fresh black, red and white mulberry fruits mg/100g on fresh weight basis

Phenolic compounds	Black mulberry <i>Morus nigra</i> L.	Red mulberry <i>Morus rubra</i> L.	White mulberry <i>Morus alba</i> L.
Protocatechuic	145.79	56.77	41.40
P.hydroxybenzoic	ND	19.19	15.94
Chlorogenic	20.37	ND	ND
Caffeic	ND	12.53	10.24
Catechin	27.73	ND	2.16
Syringic	23.69	ND	ND
Ferrulic	3.20	1.13	1.03
Chrysin	2.70	0.84	0.29
Cinnamic	1.22	7.50	0.75
P.coumaric	5.95	2.25	ND
Coumarine	4.37	2.46	1.38

Minerals

The mineral contents in three mulberry species are shown in Table (4). Results reveal differences among the mulberry species. Potassium is the most abundant element in all three mulberry species which was 1523.45, 1956.85 and 1979.35 mg/kg (on fresh weight) for black, red and white mulberries, respectively. Also, mulberry species had the high calcium content ranged from 702.26 to 866.70 mg/kg in both black and red mulberry followed by sodium and magnesium which was (108.42, 55.23) , (277.24, 51.49) and (116.20, 30.88 mg/kg) for black, red and white mulberries. Red mulberry had the highest content in iron and zinc 32.31 and 47.42 mg/kg while the lowest content showed with black mulberry. Results indicate also, that the highest content in copper was observed in white mulberry (23.32mg/kg) meanwhile black mulberry had the highest content of manganese (15.71mg/kg). These results are partially in agreement with those obtained by Koca *et al* (2008).

Antioxidant activity of different species of mulberry fruits

The antioxidant activity of different species of mulberry fruits, jams prepared by black, red and white mulberries and BHT as synthetic antioxidant are shown in Fig (1) and (2). Results indicate that BHT had the highest percentage of antioxidant activity comparing with fresh mulberry fruits or jams prepared from there fruits. The percent of BHT antioxidant activity was

92.95% meanwhile fresh black mulberry gave the highest percent of antioxidant activity 86.46% while the percents of both fresh red and white mulberries recorded 60.35 and 55.31%, respectively. On the other side, jams were prepared from three species mulberry fruits gave the lower percentages of antioxidant activity. The percents of antioxidant activity were 63.40, 56.46 and 53.52% for black, red and white mulberry jams, respectively. It was previously reported that the genotypes effects of antioxidant activity are different fruit species such as strawberries (Davik *et al.*, 2006).

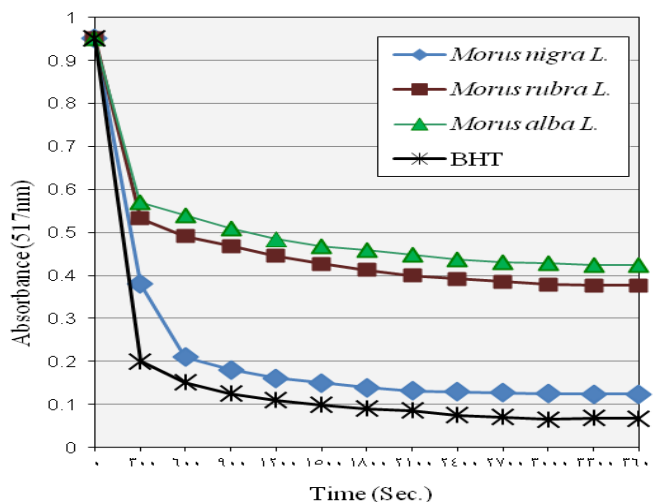


Fig. (1): Antioxidant activity in three species of fresh mulberry fruits compared with BHT (200ppm) 0.2mL/3mL DPPH extracted from 2.5gm fresh mulberry fruits by 100mL 80% ethanol

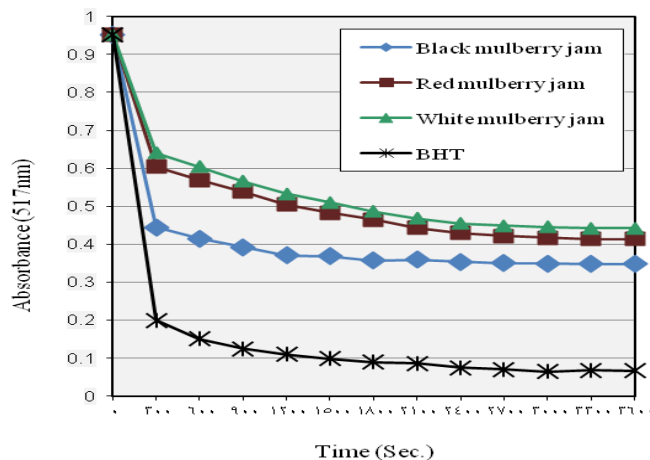


Fig. (2): Antioxidant activity in three species of mulberry fruit jams compared with BHT (200ppm) 0.2mL/3mL DPPH extracted from 2.5gm jams by 100mL 80% ethanol

Sensory evaluation of prepared jams

The sensory assessment of jams prepared by black, red and white mulberries was achieved according to scales degree (0-10) is shown in Table (5). Results indicate that black mulberry jam had the highest degree in color score (8.86) followed by white mulberry jam (8.57) while red mulberry jam recorded the lowest score (7.87). At the same time, black mulberry jam recorded the highest score in taste, odor, texture and overall acceptability, compared with other jams, which were 8.29, 8.00, 8.43 and 8.71, respectively. The best results showed with jam prepared by using black mulberry jam followed by red mulberry jam and the lowest score in jam prepared by white mulberry jam in taste, odor and texture. Moreover, in final impression black mulberry was more acceptable which recorded (8.71) while the less acceptable was the red mulberry jam which recorded (8.07).

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قسم بحوث تصنيع الحاصلات البستانية - معهد بحوث تكنولوجيا الأغذية - مركز البحوث
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تم تقييم ثلاثة أنواع من التوت المنزرعة في مصر وهي الأبيض والأحمر والأسود من حيث التركيب الكيميائي ومثبطات الأكسدة كذلك تم عمل مقارنة بين مثبطات الأكسدة لهذه الأنواع و مقارنتها بالمربي المصنعة من ثمار هذه الأنواع الثلاثة. أوضحت النتائج ان محتوى البروتين كان ١,٣٨ و ١,٢٤ و ١,١٥% علي التوالي. كما تراوحت نسبة الليبيدات الكلية بين ١,٠٨ و ١,٧٥% لكل من التوت الأسود والتوت الأحمر بينما كانت نسبة السكريات الكلية ١٢,٧٥ و ١٥,٢٥ و ١٦,٠٣% لكل من التوت الأسود والأحمر والأبيض علي التوالي. أما السكريات المختزلة فتراوحت بين ١١,٣٢ و ١٤,٠٠%. أيضاً كانت نسبة الألياف ١,٦٠ و ٢,١٧ و ٢,٢٨% للثلاثة أنواع علي التوالي والأس الهيدروجيني تراوح ما بين ٥,٤٩ و ٥,٨٠. أكدت أيضاً النتائج ان محتوى التوت الأسود من الفينولات الكلية والفلافونات والأنثوسيانين أعلى من الأنواع الأخرى بينما كان التوت الأبيض أقل الأنواع محتوى لهذه المركبات. تم التعرف علي المركبات الفينولية للأنواع الثلاثة من التوت ولوحظ ان الأنواع الثلاثة ذات محتوى مرتفع من *protocatechuic* بينما كان كل من التوت الأحمر والأبيض ذات محتوى متوسط من حامض *p. hydroxybenzoic*. وعند دراسة المحتوى من العناصر المعدنية لوحظ أن البوتاسيوم هو العنصر السائد في كل الأنواع. أيضاً هناك نسبة مرتفعة من الكالسيوم في كل من التوت الأسود والأحمر يليها المحتوى من الصوديوم والمغنسيوم. كذلك أظهرت النتائج ان التوت الأحمر يحتوي علي أعلى نسبة من الحديد والزنك كذلك اعطي التوت الأبيض أعلى محتوى من النحاس بينما كان التوت الأسود الأعلى في المحتوى من المنجنيز. وعند دراسة نشاط مثبطات الأكسدة علي كل من أنواع التوت الثلاثة وايضاً المرببات المصنعة منها ومقارنتها بمادة *butylated hydroxytoluene (BHT)* كمثبط صناعي للأكسدة حيث لوحظ ان مادة ال *BHT* تعطي أعلى معدل نشاط كمثبط للأكسدة بالمقارنة بأنواع التوت المختلفة كذلك بالنسبة للمرببات المصنعة من هذه الأنواع حيث كانت نسبة النشاط لمثبطات الأكسدة ٩٢,٩٥% كذلك اعطي التوت الأسود أعلى معدل للنشاط لمثبطات الأكسدة (٨٦,٤٦%) مقارنة بباقي الأنواع أما معدل النشاط مع كل من التوت الأحمر والأبيض فقد كانت ٦٠,٣٥ و ٥٥,٣١% علي التوالي. أما المرببات المعدة من هذه الأنواع فقد أعطت معدلات أقل للنشاط. لوحظ كذلك من نتائج التقييم الحسي ان المربي المصنعة من التوت الأسود اعطت أفضل النتائج تبعتها المربي المصنعة من التوت الأحمر وكانت أقل الدرجات مع المربي المصنعة من التوت الأبيض.

قام بتحكيم البحث

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Table (4): Minerals content of three mulberry fruit species mg/kg (on fresh weight basis)

Mulberry species	Mg	Na	K	Mn	Fe	Ca	Zn	Cu
Black mulberry	55.23±1.73	108.42±1.81	1523.45±4.68	15.71±1.57	25.27±1.46	702.26±2.98	28.17±1.33	17.07±0.76
Red mulberry	51.49±1.82	277.24±3.15	1956.85±8.22	14.89±1.26	32.31±1.67	866.70±3.95	47.42±1.16	17.31±0.97
White mulberry	30.88±1.97	116.20±1.56	1979.35±10.17	13.99±1.71	18.78±0.55	738.24±2.58	45.37±1.03	23.32±0.45

Results are presented as mean value of duplicates Mean values ± Std.Deviation

Table (5): Sensory evaluation of jams prepared from black, red and white mulberry fruits

Mulberry species	Color	Taste	Odor	Texture	Overall acceptability
Black mulberry jam	8.86 ±0.69	8.29±1.11	8.00±1.00	8.43±0.76	8.71±0.95
Red mulberry jam	7.87±0.61	8.14±0.38	7.93±0.73	8.29±0.49	8.07±0.35
White mulberry jam	8.57±0.53	8.00±0.82	7.57±0.79	8.00±0.87	8.36±0.69

Mean values ± Std.Deviation